AP13

Early Screening Test: A Routine Work to Evaluate Resistance/Susceptibility Level of Oil Palm Progenies to Basal Stem Rot Disease

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ABSTRACT

Early detection of the level of resistance or susceptibility to Ganoderma boninense *is of paramount importance for the sustainability of the oil palm industry particularly in South-East Asia. A nursery* Ganoderma *screening method has been developed and validated in collaboration with two Indonesian private companies PT PP London Sumatra Indonesia TBK (Lonsum) and PT Socfin Indonesia (Socfindo) to improve the resistance of their commercial planting material to Basal Stem Rot (BSR) disease. This early routine prenursery screening test involves the exposure of germinated seeds from different oil palm progenies with* Ganoderma*-colonized Rubber Wood Blocks (RWBs). Over the last two years, the potential testing capacities have been developed in both companies to allow the routine screening work of one hundred crosses per month. Currently, more than 1000 crosses have been tested at least two times in independent trials for their resistance/susceptibility level to* Ganoderma*. The screening method described in this paper is rapid and easy to set up in the prenursery and limits the sources of variability. Each step of the method has been previously studied, calibrated and standardized in order to reach this level of consistency. Nursery results compared with field results, showed a good correlation. This test therefore provides a method to ensure no highly-susceptible progenies are transferred to the field for commercial planting, provides a breeding tool to develop more* Ganoderma *tolerant high yielding planting material, to understand the genetics of* Ganoderma *tolerance/susceptibility, to investigate* Ganoderma *infection and as a more general tool to test other methods to control and prevent* Ganoderma.

Keywords: Elaeis guineensis, Ganoderma boninense, *basal stem rot, Oil palm, early screening test, breeding, resistance, artificial inoculation, germinated seed*

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INTRODUCTION

Genetic resistance to basal stem rot disease of oil palm (BSR), caused by *Ganoderma boninense*, is a major component of an integrated control strategy. Corley and Tinker (2003) consider that the best approach to control the disease may, in the longer term, be to develop tolerant material using nursery inoculation for screening, in much the same way as has been done for *Fusarium* wilt (de Franqueville and Renard, 1990). Sources of resistance and susceptibility to BSR have been found in field trials implemented at Socfindo in North Sumatra (de Franqueville *et al.,* 2001; Durand-Gasselin *et al.*, 2005) and by Sumatra Bioscience on Lonsum's North Sumatra estates. Field observations have proven to be consistent, given the genetic and statistical designs on which the trials were based. These results have opened up a way for using available genetic resources to improve the level of tolerance/resistance in planting material proposed to oil palm growers in BSR-risk areas. Therefore, to distinguish rapidly between sources of susceptibility and resistance to the BSR disease, the implementation of an early screening test is essential. In a previous study, our team characterised and standardised several major parameters ensuring a successful prenursery early screening test, based on artificial inoculation of the *Ganoderma* (Breton *et al.*. 2005) These included the determination of the physiological stage of the planting material, prenursery shade and temperature, incubation time and size of the inoculum source. The method of germinated seed inoculation demonstrated, for the first time, that oil palm progenies can be screened early in prenursery for their resistance level to *Ganoderma* infection. Idris *et al.* (2006) obtained similar results at the same stage which corroborates the usefulness for breeders to adopt this method of screening. At this stage, reduction in time as well as in space offers one of the main advantages for screening hundreds of progenies per year. The aim of this paper is to highlight the efficiency and the reproducibility of this early nursery screening test.

MATERIALS & METHODS

Planting material

The planting materials at the germinated seed stage were provided by the breeding units of Lonsum's Sumatra Bioscience and Socfindo's Bangun Bandar seed production section.

Inoculum source

Dikaryotic isolate of *Ganoderma boninense* used in this work was previously characterised as an aggressive isolate by Breton *et al*. (2005a, 2006). Fresh RWBs of 108 cm^3 (6x6x3 cm) were boiled for 6 hours and placed in heat-resistant polypropylene bags (2 RWBs/bag). Thirty millilitres of Potato Agar medium (PA) were added and the polypropylene bags were sealed with stoppers then covered with aluminium foil. The bags were autoclaved for one hour at 121°C, cooled overnight and then inoculated with six fragments of mycelium (25 mm^2) from a 15-day old PDA fungus culture. The polypropylene bags containing inoculated RWBs were incubated 12 weeks in the dark at 27°C with 34% relative humidity.

Inoculation method in prenursery

The inoculation of germinated seeds was performed under artificial shade (85% filtration) characterized by a temperature and a relative humidity that, during the daytime, do not inhibit mycelium growth (Nawawi and Ho, 1990) but favour the process of seedling infection in a reproducible way (Breton *et al*., 2005; 2006; Rees *et al.* 2007). The colonized RWB source was placed inside the nursery polythene bag (size 20 x 30 cm) containing soil at the bottom. Before a complete filling of polybag the depth of RWB inside the polybag was easily and rapidly standardised to obtain a distance between the germinated seed and the RWB inoculum estimate at 5

cm. The germinated seeds were planted and daily watered. Each tested cross was assessed with 5 replications of 20 germinated seeds. Twenty additional seeds per cross were inoculated at the same time for use as replacements four weeks after initial inoculation.

Disease symptoms recording

External disease symptoms (leaf symptoms and/or fruiting bodies) were recorded every two weeks after the expression of the first symptoms and then every four weeks. Twenty-two to 28 weeks after inoculation, depending on the average percentage of the standard crosses, the seedlings were split by making two longitudinal cuts in the bole and the severity of internal symptoms was assessed according to a scale established by Breton *et al*. (2006).

Data analysis

After each census, the percentage of infected seedlings was subjected to an ANOVA and Tukey tests. F value for the progeny effect was also used to measure the discriminating power of the trials at each census. The results were then expressed as an index, similar to that used for interpreting vascular wilt early resistance tests in West Africa (Renard *et al*., 1991; de Franqueville *et al*., 1995). An index under 100 indicates a higher resistance than the mean for progenies assessed in the test; an index over 100 indicates higher susceptibility. The index enables overall comparisons and makes it possible to rapidly and clearly establish a susceptibility range, provided standard crosses with a wide range of resistance/susceptibility are involved in the trials. Comparing index results between trials is therefore potentially misleading without the use of standard crosses.

RESULTS & DISCUSSION

Most of the inoculation techniques described to-date (Khairudin *et al.*, 1991; Sariah *et al*., 1994; Idris *et al.*, 2004; Rees *et al*., 2007) used at least 3-month-old seedlings, with a testing period of several months. The screening method reported in this study has the result in a drastic reduction in the testing period and the early differentiation of resistance/susceptibility of tested progenies to BSR disease (Fig. 1 and Fig. 2). These results are a consequence of previous research on the characterization and the standardization of several major parameters involved in the infection process (Breton *et al*., 2005). More recently, some of them have been confirmed by Rees *et al.* (2007).

At the end of each screening trial, internal symptoms were recorded and analysed in the same way as external symptoms. An average around 5% of infected seedlings had no external symptoms of the disease. This result is similar to the field observation where 15% or more, according to the planting location, of mature palms are infected but do not exhibit external disease symptoms. This difference of percentage external/internal symptoms with nursery results is probably only because of the physiological stage of the seedlings used in screening tests. Even if the percentage of infected seedlings scored in nursery with internal symptoms was higher than the percentage of external symptoms, the ranking of the progenies is not significantly modified. The Pearson correlation coefficient was 0.94 (average value from 25 randomised screening trials) and from testing more than 1000 crosses, no inversion of the resistance level has appeared between data collected from external and internal symptoms.

The “discriminating power” of this screening test was estimated by the F-value of the progeny effect computed by the ANOVA (Fig. 3). Before 20 weeks, infection rates were variable (not stabilized) between progenies whose infection started early and progenies whose infection appeared later. Twenty to twenty two weeks after inoculation, F-values did not vary extensively and relative variation between progenies had stabilized; which corresponds to an average percentage of infected standard crosses of around 30%. There may be potential to further reduce the length of nursery

screening trials as the main objective is to broadly discriminate between susceptible and tolerant crosses. Since the nursery screening trials have not revealed progeny x time interactions it may not be necessary to continue the trial until maximum variation between progenies is determined to have a highly fast and efficient selection tool.

A set of standard crosses (both susceptible and tolerant) have been selected to allow trials linkage and the combined analysis of trial results. Table 1 show some standard crosses previously characterised in terms of their resistance/susceptibility to *Ganoderma*. For this evaluation, each standard was tested several times in independent trials. These results demonstrated also the reproducibility and the consistency of the test. Currently, all the screening trials are linked by standard crosses. This inter-connection of trials is done by computing at the end of each new test a corrective coefficient applied to the raw index of every cross and which makes the corrected indexes for standard crosses as close as possible to what was observed in previous tests (Table 2). This corrective coefficient does not change the ranking of tested progenies but improves the consistency of data between trials and then, the accuracy of the test. It is necessary to assess the extent to which there are progeny x trial interactions before simply ranking of progenies for *Ganoderma* tolerance or susceptibility. The main data bank was established with this new corrective index. The distribution of the resistance/susceptibility to *Ganoderma* is continuous (Fig. 4) and indicates that more than one or two major genes are involved in providing tolerance/resistance.

The relation with field observation was clearly established for some standards and tested crosses (Table 1 and 3) and also *via* breeding pedigrees. The major challenge in the interpretation of field trial results is the qualitative and quantitative variability of the inoculum sources, the multiple field trial locations and the range of statistical field trial designs used. A positive correlation between nursery and field has been demonstrated for several tens of crosses and the nursery results were obtained from more than 1000 tested progenies are coherent in term of genetic origins. Nursery results compared with field results, recorded in natural condition of infection, showed that with the test, no highly-susceptible progenies would have been planted (Table 1 and 3).

An important parameter to keep in mind was the high genetic variability of *Ganoderma* isolates. Until now no specific isolate x progeny interactions have been identified. However, work to collect new isolates and to test them in progeny x isolate trials continues as such interactions may be identified in the future. In this work, an isolate previously characterised as aggressive was used. The more resistant crosses tested to with his isolate could be screened with an even more aggressive isolate. This sequential screening of oil palm material permits the release of highly productive commercial material which is less resistant to BSR but which could be planted in area of low potential *Ganoderma* infection.

This efficient nursery screening method has also been used to rapidly the efficiency of fungicides and *Ganoderma* antagonists such as *Trichoderma* isolates before establishing field trials.

CONCLUSION

An early and rapid screening test of oil palm progenies to BSR disease has been developed and validated by using planting and breeding materials from two Indonesian private companies - PT PP London Sumatra Indonesia (Lonsum) and PT Socfin Indonesia (Socfindo). The results are both consistent and reproducible and correlated to field observations. Moreover, this prenursery test using germinated seed allowed the screening of several hundreds crosses per year and is highly asset valuable to plant breeders. There are a range of uses of this nursery screening tool beyond simple screening out of susceptible progenies e.g. genetic studies, potential of antagonisitic fungi and fungicides. Over two years, the potential testing capacities were increased in both companies *via* the setup of two new *Ganoderma* screening laboratories (with specific nurseries, 6000 m^2) allowing the screening of 100 to 150 progenies per month (Fig. 5).

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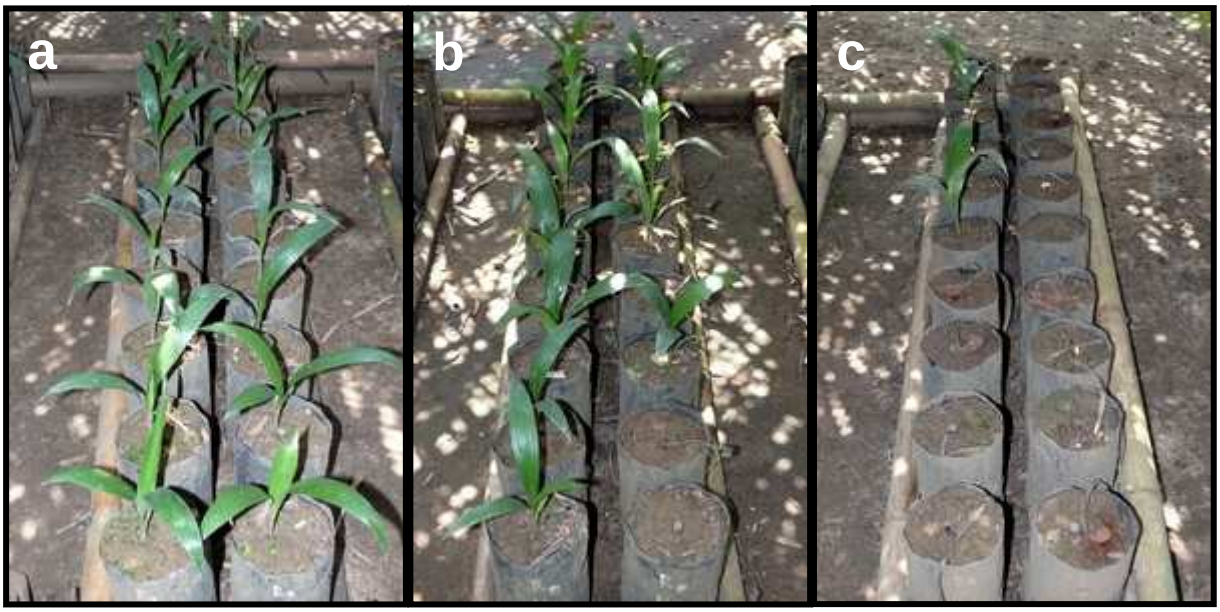

Figure 1: Disease expression observed 22 weeks after artificial inoculation of germinated seeds by *Ganoderma boninense*. a: resistant progeny; b: intermediate progeny; c: highly-susceptible progeny.

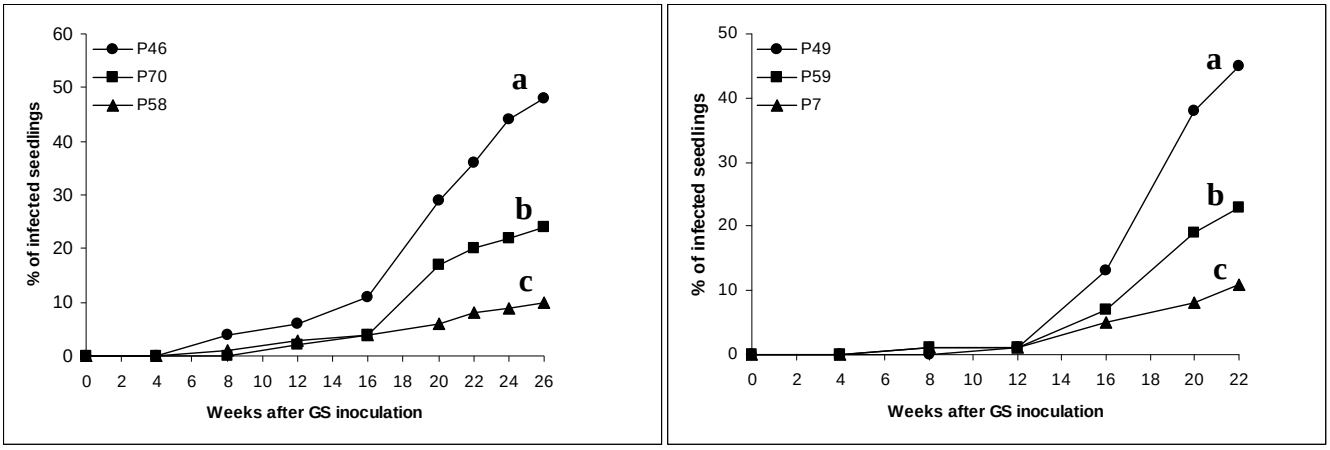

Figure 2: Kinetics of BSR development following artificial contamination of oil palm germinated seeds by *Ganoderma boninense*. For each trial (a) and (b) three independent progenies characterized by different levels of susceptibility to the BSR were selected among the 100 crosses tested in these trials. Curves with a common letter are not statistically significant by Tukey test at p=0.005.

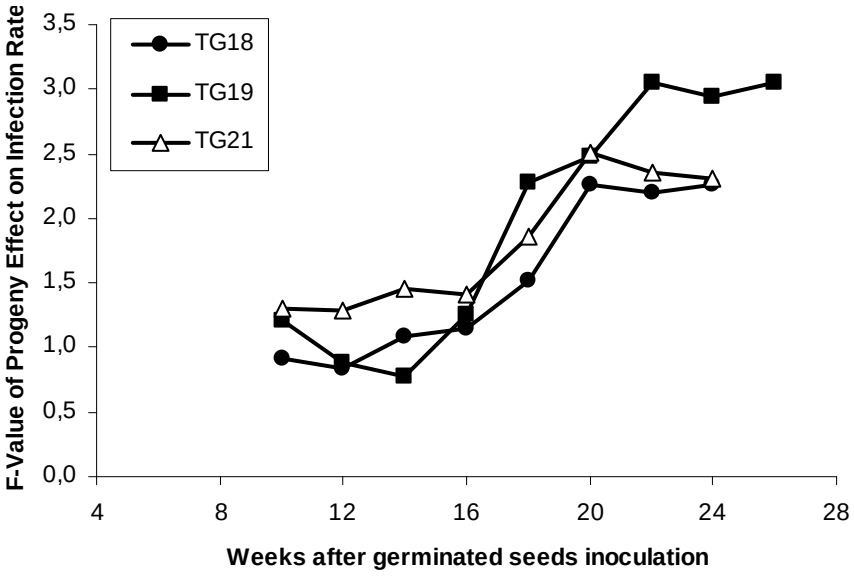

Figure 3: Evolution of F-value for the screening tests TG18, TG19 and TG21 (for example) during the period of recording the external disease symptoms.

Standards crosses		Number of independent tests performed	Index distribution between independent tests (base 100)		Average Index (30%)	Data summary < 100 <
Categories	Codes		Lower than 100	higher than 100		
Susceptible	S1	3	0	3	156	0-3
	S2	11	3	8	123	3-8
	S3	8	0	8	142	0-8
	S4	3	0	3	156	0-3
	S5	4	0	4	134	0-4
	S6	3	0	3	154	0-3
	S7	4	0	4	155	0-4
	S8	7	3	4	120	3-4
	S9	11	3	8	114	3-8
	S10	9	3	6	114	3-6
Medium	M1	4	2	2	97	2-2
	M2	6	2	4	109	2-4
	M3	3	2	1	94	2-1
	M4	4	2	2	98	2-2
	M5	14	8	6	95	8-6
	M6	20	10	10	100	10-10
	M7	4	2	2	108	2-2
	M8	6	3	3	99	3-3
	M9	6	3	3	102	3-3
	M10	5	3	2	96	3-2
Tolerant	T1	5	5	0	45	5-0
	T2	7	6	1	75	6-1
	T3	7	6	1	78	6-1
	T4	4	4	0	77	4-0
	T5	16	14	2	81	14-2
	T6	10	10	0	56	10-0
	T7	6	5	1	66	5-1
	T8	9	9	0	62	9-0
	T9	11	10	1	63	10-1
	T10	8	7	1	80	7-1

Table 1: Index average and distribution of several standards crosses tested in independent nursery trials.

Screening tests	Corrective coefficient
TG001	1,0718604
TG002	1,0362016
TG003	0,9911608
TG004	1,0957004
TG005	1,0550731
TG006	0,9249014
TG007	0,9704672
TG008	0,9231358
TG009	1,0332698
TG010	1,0425663
TG011	1,1319809
TG012	1,0404507
TG013	1,1435525
TG014	0,8924268
TG015	0,8676366
TG016	0,9404257
TG017	1,0082759
TG018	1,0056641
TG019	0,9907746
TG020	1,0536897
TG021	1,0397795
TG022	1,0584877
TG023	1,0286684
TG024	1,0810396
TG025	0,880657
TG026	0,9282029
TG027	0,9918462
TG028	0,9193883
TG029	0,92468
TG030	1,0888646

Table 2: Example of corrective coefficient applied to the raw index at the end of the trial. This coefficient is computed thanks to the presence of common crosses (standards) between each independent trial.

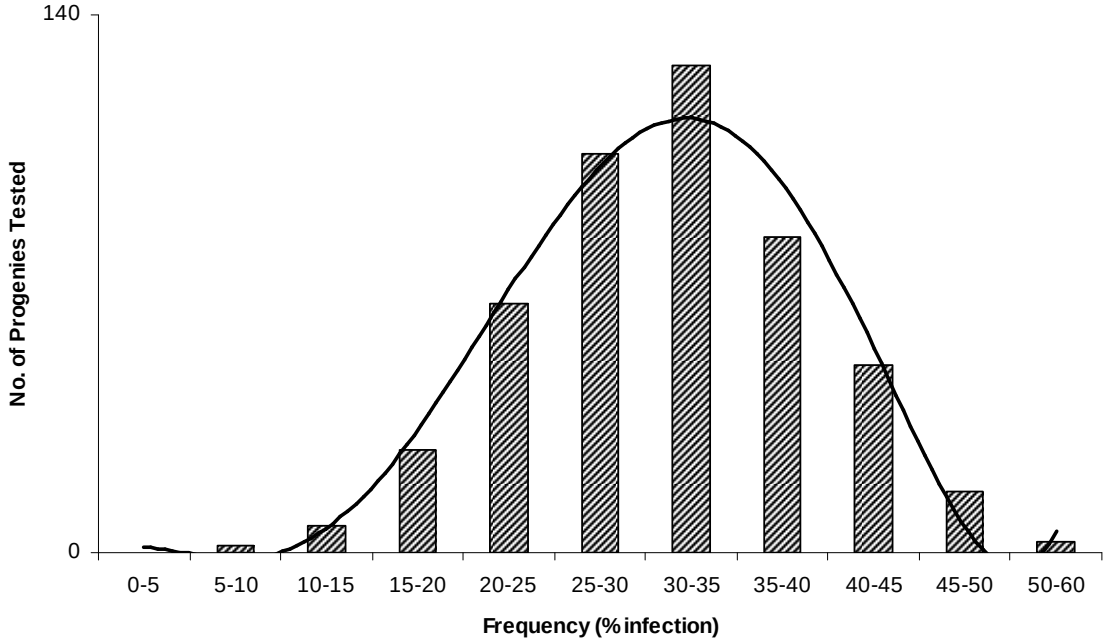

Figure 4: Example of distribution of the resistance/susceptibility to *Ganoderma* within a genetic population of oil palm. A total of 482 crosses representative of the population were tested in several independent trials. The crosses were grouped arbitrary according to the percentage of infection. The repartition of the number of crosses per groups seems to follow a normal law.

Progeny code	Field status [1]	Number of trials [2]	Index average	Index distribution		Test status
				Id [3]<100	Id>100	
1	Intermediate	4	86	3	1	Resistant to intermediate
2	Susceptible	11	123	3	8	Susceptible
3	-	4	79	3	1	Resistant
4	Susceptible	4	122	1	3	Susceptible
5	Resistant	5	75	5	0	Resistant
6	-	4	75	3	1	Resistant
7	Intermediate	4	97	2	2	Intermediate
8	Intermediate	6	109	2	4	Intermediate to susceptible
9	Susceptible	8	141	0	8	Susceptible
10	Susceptible	5	124	1	4	Susceptible
11	Intermediate	4	101	2	2	Intermediate
12	Resistant	5	46	5	0	Resistant
13	Intermediate	12	89	9	3	Resistant to intermediate
14	Resistant	5	77	5	0	Resistant
15	Resistant	6	80	5	1	Resistant
16	Resistant	9	84	7	2	Resistant to intermediate
17	Susceptible	7	113	2	5	Susceptible
18	Exp. susceptible	8	100	4	4	Intermediate to susceptible
19	-	5	74	4	1	Resistant
20	Susceptible	5	105	2	3	Intermediate to susceptible
21	-	6	127	2	4	Susceptible
22	-	5	76	5	0	Resistant
23	Exp. resistant	4	65	4	0	Resistant
24	-	4	112	1	3	Susceptible
25	Exp. resistant	5	77	5	0	Resistant
26	-	4	95	2	2	Intermediate
27	Exp. resistant	6	70	6	0	Resistant

[1] : provisional field status, subject to evolution
[2] : number of nursery trials in which the progeny have been tested
[3] : index

Table 3: Example of relation for some DP crosses between results from field observations with results from nursery screening test.

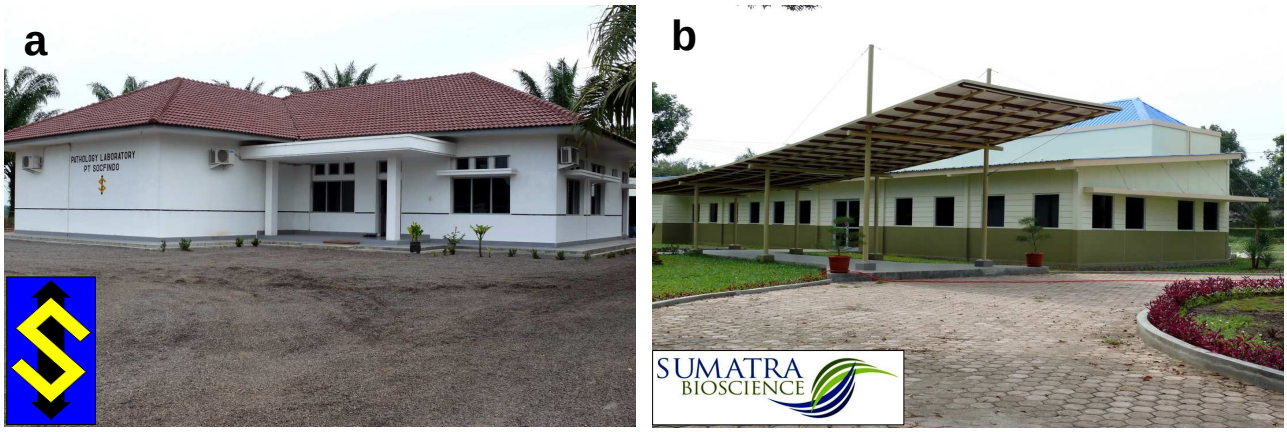

Figure 5: Implementation of *Ganoderma* screening units, PT Socfindo (a) and PT PP Lonsum (b). The two new laboratories have a screening capacity in routine work of 100 to 150 crosses per month.